OBSERVATIONS ON THE METABOLISM OF *SARCINA LUTEA*. II

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In the preceding paper the effects of anærobiosis on this organism and the influence of glucose on its oxygen consumption have been reported. It seemed desirable to extend these observations to the influence of lactic acid, as one of the carbohydrate intermediates, and also to study the influence of the respiratory catalyst, methylene blue, and of the inhibitors, carbon monoxide and cyanide. The results of these experiments have been surprising in several respects. The methods used were exactly as previously described, and all experiments were carried out at 22° C.

Results

1. *Water and Saline Suspensions.*—This series of 34 experiments in water shows an average Q_{O_2} of 2.5 during the relatively steady period five hours after the start of readings (Table I). This is in good

Table I

Q_{O_2} in Water Suspension

Initial value Q_{O_2}	Later value	
	Hours	Q_{O_2}
8.1 (4) *	5	3.5
9.3 (4)	5	3.3
7.2 (4)	4.5	2.4
7.5 (4)	4 +	2.5
8.5 (2)	4.5	1.8
5.3 (4)	5	2.1
5.8 (2)	4.5	2.0
5.1 (3)	3.5	2.2
4.7 (2)	6	1.2
6.1 (1)	5.5	3.0
8.0 (4)	5	2.3
? 1.4 (2)	5	0.8
? 1.8 (3)	3	1.2

* Bracketed number represents number of separate runs.

agreement with the value of 2.6 for the first, smaller series. The temperature was slightly higher in this series but the average was

taken at a somewhat later time. The initial Q_{O_2} average is 7.1, a value depending on the conditions of preparation, as previously discussed. Two experiments at 37° C. compared with 22° C. gave a Q_{10} of approximately 1.7.

Barron and Harrop (1929) found a marked effect of crowding on the Q_{O_2} of white blood cells, the respiration per unit of material falling as more and more concentrated suspensions were used. The possibility of a similar effect in these experiments was tested by varying the density of the suspension from the start or by suddenly diluting a water suspension during a run by tipping in more water. No change was observed, so that this factor may be excluded. It might well depend, in the case of the blood cells, on limitation of motility of the undulating membrane in the heavier suspensions.

The effect of NaCl in concentrations up to 1.1 per cent was determined, as a control of osmotic and ion effects with other reagents. The lower concentrations were without effect, and even the highest used gave a maximum decrease of 20 per cent of the Q_{O_2} as compared with water, and this only occasionally. Further addition of one part to ten of M/15 phosphate buffer was likewise of no consequence for the oxygen consumption; nor was the pH, between 7 and 8. When salt solutions were tipped into a water suspension of respiring bacteria, a small brief acceleration was often observed, after which the original curve was resumed (Fig. 3). Whether this is a true momentary "stimulation" or some small experimental error has not been further investigated; in either case the results serve as a control for the observed changes when other substances are tipped in.

2. *Methylene Blue.*—Addition of methylene blue (0.2 per cent or 0.1 per cent methylene blue "for vital staining") to a water or buffered saline suspension always raised the Q_{O_2} by about one hundred per cent; from an average value of 1.9 to one of 3.9 in seven experiments, an increase of two. The increased oxygen consumption tends to fall off with time but remains above that of water for several hours. The effect of methylene blue plus glucose, sodium lactate, or sodium cyanide will be considered with those substances.

3. *Glucose.*—The effect of glucose addition has been followed somewhat further than in the preceding paper, by tipping a glucose solution into a water suspension of the cocci during a run. This makes it possible to obtain the entire glucose effect, since no time is lost after its addition, during which readings cannot be made. Table II sums up all the results with plain glucose. The average Q_{O_2} of 2.2 before tipping in the sugar rose to 10.5 for the first period (one-half to one hour) after tipping and then fell. At three and one-half

TABLE II

Respiration in water before addition	Added		Maximum after addition	Later value	
	Glucose	Methylene blue			
Q_{O_2}	Per cent		Q_{O_2}	Hours	Q_{O_2}
* 2.3	.5	+	13.6	6	1.3
* 2.5	.5	−	10.4	6	4.1
2.1	8.8	−	15.0	5	4.5
2.0	0.4	−	9.7	4.5	4.5
1.9	0.2	−	9.3	2	8.5
2.5	0.8	+	14.6	2	14.4
2.3	0.8	−	9.2	2	9.7
2.2	0.2	+	19.2	2 (18)	4.9 (0.5)
2.3	0.2	−	10.0	2 (18)	4.4 (1.2)
2.3	0.8	+	18.2	2 (18)	13.2 (0.3)
2.3	0.8	−	12.2	2 (18)	8.2 (0.5)

* Parallel runs.

hours the average Q_{O_2} was six. The maximum reached was independent of glucose concentration (though 8.8 per cent glucose gave the greatest increase observed), but the subsequent fall was somewhat slower with larger amounts than smaller.

The total excess oxygen consumed by the bacteria due to the addition of glucose can be determined by subtracting the Q_{O_2} curve in water from that with glucose added. In at least two cases this has been definitely greater than the amount required to completely oxidize all the added glucose, which suggests a "specific dynamic action" of this substance. Confirmatory evidence is obtained from the R.Q. findings, discussed previously, and from the further observation that suspensions carried for 24 hours in glucose solutions have a lower Q_{O_2} than similar ones in water, as if the higher rates at first had more completely exhausted the cell reserves. Evidence of a stimulating action of glucose in nitrogen has also been previously presented. All these observations indicate a dynamic action of glucose though they are hardly extensive enough to permit sweeping conclusions.

Methylene blue plus glucose leads to a greater initial increase of oxygen consumption than the sum of their separate effects, but this excess becomes less with time (Fig. 1). Thus, in four parallel experi-

ments, the average maximum in glucose was 11.2, in glucose plus 0.2 per cent methylene blue, 16.5. Seven hours later the values were 4.7 and 5.8, and the next morning, in all cases followed, the values were higher in the suspensions in glucose without the dye. This diminishing or reversing of the methylene blue increase with time is

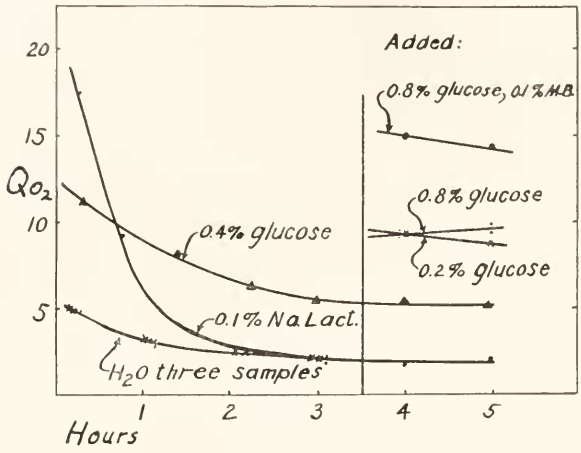

FIG. 1.

similar to the results with water solutions of the dye or of glucose alone. The absolute increase of Q_{O_2} when methylene blue is added to water suspensions is 2.0, but the percentage increase is about one hundred. When the dye is added to glucose suspensions, the absolute increase is over 5, the percentage increase fifty. The interpretation comes to mind that these two substances act at successive steps in the chain of oxidative reactions, thus leading to a product of their separate effects, rather than concomitantly, which should give only the sum. Much evidence exists that this is indeed the situation. The recent findings of Warburg, Kubowitz and Christian (1930) and Wendel (1930) indicate that reduction of methylene blue may lead to peroxide formation with cytochrome, which in turn oxidizes other substances (as lactic acid to pyruvic acid), and leaves the iron in the Fe^{+++} stage. The second reaction might well depend, if only indirectly, on the amount of oxidizable material (glucose) present. The findings with lactate additions, however, are not entirely in harmony with this view.

4. *Sodium Lactate.*—The greatest rates of oxygen consumption obtained resulted from the addition of lactate to water or saline suspensions. The maximum Q_{O_2} after tipping averaged over ten times that just before, and a more than twenty-fold increase has been observed. Though the maximum Q_{O_2} varied in different runs from

17 to over 50, the variation was low in any one run and not related to the concentration of lactate added (Fig. 2). (In one experiment a regular rise in maximum with concentration was observed.) The subsequent course of the extra oxygen consumption, on the other

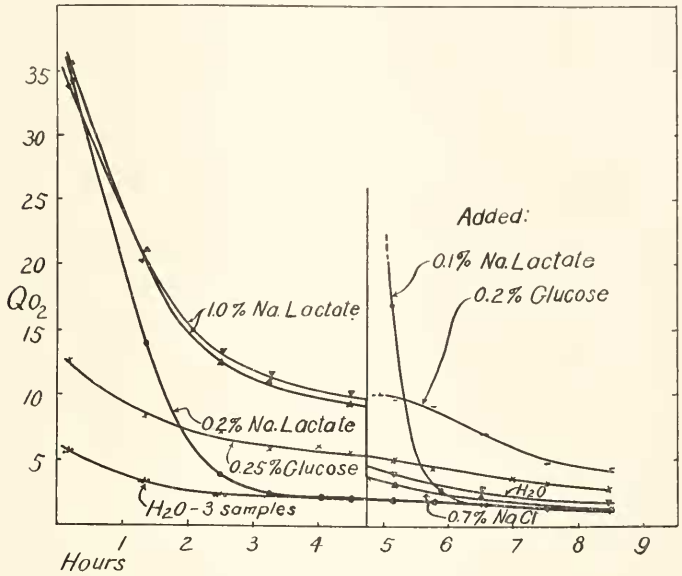

FIG. 2.

hand, was intimately dependent on this concentration (Figs. 2 and 3, Table III). Thus, the average of a series of twelve experiments gave a Q_{O_2} of 2 in water, and a maximum of 23 after sodium lactate was tipped. Five hours later the Q_{O_2} values for various concentrations were:

0.05% = 1.4; 0.1% = 2.5; 0.25% = 3.0; 0.55% = 6.0; 1.1% = 7.0.

Such a falling off with time, of the lactate effect, is due in part to the actual removal of lactate, but cannot be accounted for entirely on this basis. A few hours after the addition of 1.0 per cent lactate ions, the Q_{O_2} will have fallen to, say, one-half of the maximum. It can be inferred (see below) with considerable certainty that over 0.5 per cent lactate is still present. It has been found, however, that when 0.5 per cent lactate is freshly added to a water suspension, the Q_{O_2} reaches the same maximum as when 1.0 per cent is added; so that the falling off in the first case must be complicated by other factors. An occasional finding, which also indicates the complexity of the system, is an immediate increase of respiration rate after lactate

(or glucose) addition followed by a further rise for an hour or more before the usual fall sets in. This appeared oftener with the more concentrated additions, as if the strong lactate partly inhibited respiration until some was metabolized away.

The amount of lactic acid actually burned may be estimated from the excess oxygen consumed, on the assumption that just this extra oxygen is all used to completely oxidize the acid. In most experiments 0.2 cc. of a 0.2 per cent solution of lactate (as the sodium salt)

TABLE III

Respiration in water before addition	Added		Maximum after addition	Later value	
	Sodium lactate	Other substance			
Q_{O_2}	Per cent		Q_{O_2}	Hours	Q_{O_2}
3.5	.2	Methylene blue	48	7	1.5
4.3	.2	Methylene blue	52	7	0.5
4.5	.2	—	65	7	3.0
2.6	.2	Methylene blue	17	6	1.1
2.5	.2	—	20	6	1.9
1.8	2.2	In water	17	4.5	8.3
	1.1	In NaCl, PO_4 buffer, pH7	18	4.5	7.4
1.4	1.4	Same, pH8	19	4.5	8.2
2.2	2.2	—	29	5	5.5
2.0	0.5	—	28	5	3.0

was tipped into 0.2 cc. of the bacterial suspension, giving a final concentration of 0.1 per cent. This quantity of lactic acid, 0.0044 millimols, would require 298 cu. mm. of oxygen for full oxidization. The values actually obtained for the extra oxygen consumed (the area under the Q_{O_2} curve with lactate present less that with lactate absent —the two curves usually joining some hours after the start) were:

210, 250, 200, 270, 310.

The average, 250, is somewhat less (15 per cent) than theoretical for the complete burning of all the lactate. This is probably a technical error due to failure to rinse all the fluid from the side bulb into the main chamber, with the consequent exclusion of some of the lactate from the bacteria. Unfortunately, the quantitative possibilities were not in mind when the experiments were carried out, and the usual to and fro pouring was ordinarily omitted because of the danger of

spilling alkali—a narrow opening to the side-bulb made rather severe tapping necessary in some cases. In general, the agreement is good enough to indicate complete oxidation of small amounts of lactic acid.

With larger quantities, that is, more concentrated solutions, oxidation was not complete even in twenty-four hours—when the extra oxygen consumption had ended. Thus for a 1.0 per cent solution 1500 cu. mm. of oxygen would have been required for full oxidation. The excess oxygen totalled over 700 cu. mm., and the total oxygen used (*i.e.*, the lactate Q_{O_2} curve not corrected by the water control) after the lactate addition only about 900 cubic millimeters.

The possibility at once suggests itself that the falling off of the excess respiration, and the failure of complete oxidation of the larger amounts of lactic acid are alike due to the accumulation of injurious reaction products. A rise in pH, in particular, must result from the conversion of lactate ion into CO_2, two-thirds of which would leave the bacterial suspension and be absorbed in strong alkali in the inset of the vessel, while one-third would remain to combine with the free Na^+ ions, giving $NaHCO_3$ in place of $NaC_3H_5O_3$. Such a pH change is not, however, a critical factor; for suspensions buffered at pH 7 or 8 show the same Q_{O_2} curve with added buffered sodium lactate as do

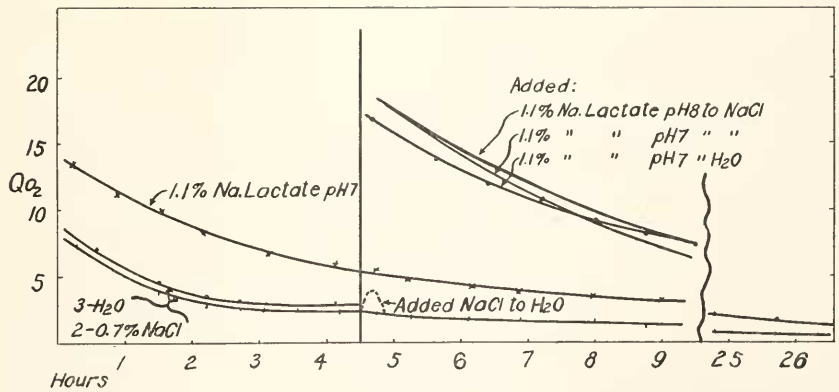

FIG. 3.

water suspensions (Fig. 3, Table III). Also, in one series, a phosphate buffer at pH 5.9 was added to the suspensions in 1/10 and 1/5 concentrations. After a long run with much added lactate, the 1/10 buffer was exhausted and the suspension alkaline to litmus, while that with the stronger buffer was still faintly acid. The Q_{O_2} curves, however, were almost perfect duplicates. Further, it is doubtful if any kind of inhibiting end-products can be involved. If such were the case,

dilution of a suspension which has had lactate added some time before should cause the fallen Q_{O_2} to again rise to some extent. Actually the reverse is found; dilution promptly lowers the Q_{O_2} still further (Fig. 2).

Finally, it is interesting to note the effect of a double addition of lactate (Fig. 4). A suspension in 0.3 per cent lactate at an early stage (1–2 hours) and with a markedly increased respiration is in no way affected by the further addition of one-third its volume of 0.6

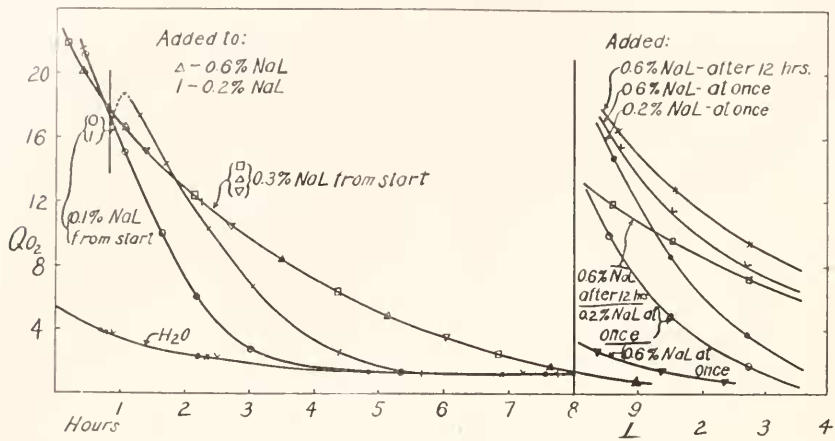

Fig. 4.

per cent lactate. There is no immediate increased oxygen consumption nor any delay in the subsequent fall. If the second portion of lactate is added at the end of nine hours, when the original lactate action has ended and respiration is the same as in a water control, there is some increased consumption but much less than in the control. In one experiment, for example, the Q_{O_2} in both water and lactate suspensions had fallen to 1.5. When new lactate was added to the lactate suspension, this value rose only to 3, while the Q_{O_2} of the water suspension rose to eighteen. If the lactate addition is delayed another 12 hours, however, the water and lactate suspensions being shaken in the usual manner in manometers, the difference is much less marked. Thus, in the same set of runs, lactate added to the water suspension 21 hours after the start again gave a Q_{O_2} of 18, and added to the lactate suspension, one of 12 instead of three. Obviously, a second lactate addition has no effect when added soon after a first one, some effect when added after the initial increase in respiration is past, and close to a maximal effect when added the following day.

When the experiment is carried out with 0.1 per cent lactate and 0.2 per cent lactate added later, the time relations are altered. With

these dilutions, a second addition has some effect even two hours after the first. Thus, in one case (Fig. 4), the fall in respiration was delayed an hour or more and some actual increase may have been present at once after the second addition. It is interesting to note further that the actual Q_{O_2} at the moment the addition was made was the same as that of the suspension in 0.3 per cent lactate where a further addition was ineffective. After nine hours, four hours after the respiration of the lactate suspension had become identical with that of the water control, the further addition of 0.2 per cent lactate to each gave the following Q_{O_2} values: water suspension, 19; lactate suspension, fourteen.

These observations strongly suggest that the falling off in rate of oxygen consumption with time is due less to accumulation of some retarding factor than to a temporary exhaustion of some required one. Certainly neither oxygen nor lactate are lacking, and no retarding substance accumulates as the ultimate product. Either some intermediate product is formed rapidly and but slowly further altered, so as to act as a temporary obstacle to the first reaction; or the oxidative mechanisms of the cell are somehow run down by an excessive load and are only gradually restored to the equilibrium state. The effects of sudden dilution are more in accord with the second possibility.

The effect of methylene blue combined with lactate is of interest in this connection. When, in comparable experiments, buffered solutions of lactate alone or with methylene blue are tipped into a water suspension, the maximum Q_{O_2} is consistently greater with the dye absent (Table III). In eight experiments with 0.1 per cent sodium lactate and 0.1 per cent methylene blue the maximum Q_{O_2} averaged 34; in four without the methylene blue, forty. In every run the depressant action of the dye was seen, decreases ranging from 10 to 30 per cent. At two to three hours the curves approached or even crossed for a short time, but at about six hours after the tipping the values were: with methylene blue, $Q_{O_2} = 0.9$, without, $Q_{O_2} = 2.5$.

Methylene blue has, to sum up, a marked early accelerating action on the respiration of *Sarcina* suspensions in water or in glucose solutions. After some hours the rate of oxygen consumption falls to that of control suspensions and is ultimately depressed. With lactate solutions, the depressant action of methylene blue is present at the start, and, in percentage, becomes more marked with the passage of time.

It may be pointed out that for equimolar amounts of glucose and sodium lactate added, the time course of extra oxygen consumption is very different. The glucose leads to a moderate prolonged rise,

the lactate to an intense brief one (Fig. 2). This would be in harmony with, though of course not necessitating, a slow split of the glucose molecule to lactic acid or a closely related intermediate followed by a rapid oxidation of this latter. Barron's view (1930) that methylene blue catalyzes primarily the initial splitting of glucose and not the later reactions would then fit the observed results fairly well. The main difficulty with such an interpretation lies in the failure to obtain an accumulation of lactic, pyruvic or other acid stronger than carbonic under anaerobic conditions, as shown in the preceding paper.

Finally, simultaneous addition of glucose and sodium lactate leads to the sharp maximum of lactate followed by the less intense enduring glucose effect. There does not appear to be summation of the two effects—the Q_{O_2} follows the higher single curve (lactate alone or glucose alone) at any time. No separate experiments were performed with optically active lactic acid, the racemate serving in these tests. Since, however, all added lactate can be burned and the Q_{O_2} curve after lactate addition gives no evidence of a discontinuity, it seems probable that both the *d* and *l* forms are easily utilized by this organism.

5. *Thioglycollic Acid.*—A few experiments, made in another connection, have shown that this substance in 0.2*M* concentration doubles the oxygen consumption.

6. *Sodium Cyanide.*—Cyanide, in concentrations up to *N*/100 or a little stronger does not inhibit the oxygen consumption of *Sarcina lutea*. This is true for the usual respiration of suspensions in water, saline, or phosphate buffer, as well as for the increases evoked by methylene blue, lactate, or glucose (Fig. 5). The presence of cyanide

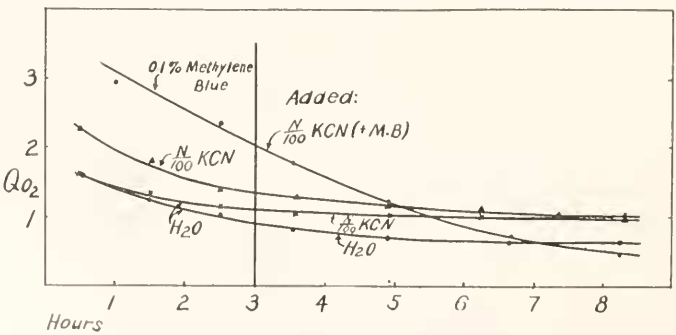

FIG. 5.

appears, in fact, to slightly increase the Q_{O_2} or at least to diminish the rate of fall with time. The average Q_{O_2} of five experiments in water was 1.1 at three hours; in *N*/100 NaCn it was 1.4. The possibility of HCN distilling from the side bulb to the main suspension

prematurely, and so masking a real effect, is excluded. In $N/30$ cyanide, respiration is depressed 1/2 to 2/3, and in $N/10$, 2/3 to 3/4. Rubinstein (1931) has confirmed the absence of cyanide inhibition with water suspensions.[1]

A much less complete absence of cyanide inhibition has been described by Emerson (1927) for the alga *Chlorella*, for in the presence of sugar 10^{-4} M cyanide gives 50 per cent inhibition. Lund (1918) has claimed a cyanide insensitivity for *Paramecium*, though this has not passed unchallenged (Hyman, 1919).[2] Pitts (unpublished) has found *Colpidium* to be but slightly sensitive to cyanide. Burnet (1927), studying growth and oxygen consumption of a number of bacterial types under the influence of cyanide, found them divisible into two groups. Most showed a usual degree of sensitivity, but some, as streptococci, were insensitive to cyanide. These groups were also different in their behavior with hydrogen peroxide. Even for vertebrate tissues a complete inhibition of respiration by cyanide is not the rule. $N/100$ does not inhibit the respiration of frog nerve by more than 80 per cent (Gerard, 1930), and Dixon and Elliot (1929) obtained similar results with a variety of mammalian tissues, though Alt (1930) and Warburg (1930) sharply criticize their work. The objections raised certainly do not apply to the present results. *Sarcina lutea* apparently carried on a fairly typical aerobic metabolism, using oxygen freely to burn the usual organic molecules. The almost complete insensitivity of its respiratory catalytic system to cyanide would seem to preclude a too broad generalization as to the ferroactive nature of oxidative enzymes.[3] The action of carbon monoxide is likewise atypical.

7. *Carbon Monoxide.*—Carbon monoxide containing 5 per cent

[1] Working with descendants of the original strain, over a year later, Barron obtained definite inhibition of respiration by cyanide. I repeated my original experiments, using the identical techniques previously employed, and also found inhibition was now present. In water suspensions $N/100$ cyanide gave depressions of 50 per cent or more, in glucose even greater ones. The depressions were, however, of short duration and respiration returned to normal in an hour and sometimes became excessive later. Presumably the organisms had altered in the interim due, perhaps, to unknown differences in the culture conditions.

[2] This has now been confirmed. Gerard and Hyman, *Am. Jour. Physiol.*, in press.

[3] It may be mentioned here that Dr. E. S. G. Barron and I have demonstrated by means of the spectroscope that cytochrome is present in these cells. Further, it is changed easily between the oxidized and reduced states by bubbling in oxygen or nitrogen. In the presence of $N/100$ KCN, however, the pigment remains reduced even in a stream of oxygen. This appears as evidence that cytochrome is not an essential link in the chain of respiratory substances of this cell, since respiration proceeds as normally after its oxidation is blocked. We are further investigating the oxidizing mechanisms of *Sarcina*.

oxygen was used in all experiments and the thermostat covered to exclude daylight. A 200-watt tungsten bulb, immersed in the white-walled thermostat, within 2 to 10 inches of the manometer chambers, was turned on at intervals of 20 minutes to 1 hour.

Water suspensions of *Sarcina* respired alike in air and in the monoxide mixture. Light had no obvious effect on either. When glucose (1.0 per cent) or lactate (1.0 per cent) was added to such water suspensions, an inhibiting action of the CO appeared. In the case of glucose this was slight, the increased oxygen consumption in CO being over three-fourths that in air. With lactate, the inhibition was marked, the increase in monoxide being less than one-fifth that in air, but inadequate oxygen may have contributed to the effect. In both cases, glucose or lactate added, when the carbon monoxide

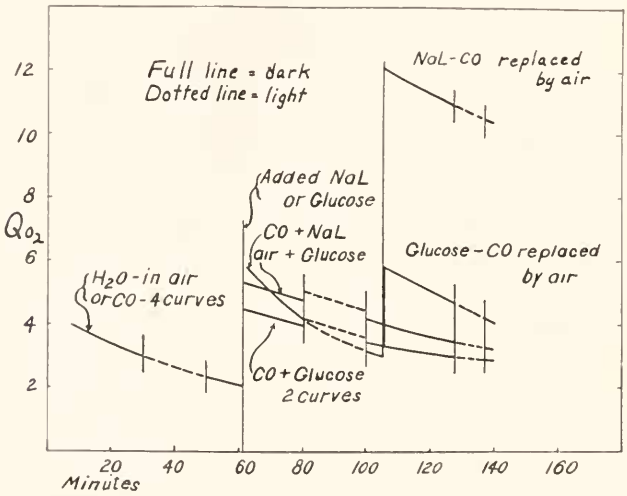

FIG. 6.

mixture was later replaced by air, the full rate of oxygen consumption promptly appeared. The presence of light, of the intensity used, had no reversing action on the inhibited lactate oxidation. There was a suggestion of such an effect in increasing the respiration of a suspension in glucose in the presence of CO, but an entirely similar augmentation appeared also in air (Fig. 6). Rubenstein (1931) has found that light increases the respiration of *Sarcina lutea* at this temperature.

DISCUSSION

These results raise several interesting questions regarding the chemical dynamics involved. Q_{O_2} is a measure of reaction velocity, yet this is independent of the concentration of the primary reactants

over a wide range. Thus 0.05 and 1.1 per cent lactate give the same maximal rates, but both rates decline following the maximum. For the lower concentrations, at least, the rate must fall with decreasing amount of substrate. Probably at sufficiently low initial concentrations, the maximum reached would vary with concentration, until an asymptotic value had been reached, beyond which concentration has no effect. The critical concentration of lactate cannot be over 0.05 per cent, but may be very close to this. The decline in rate after the maximum, when considerably larger amounts of lactate are present, cannot, as in the previous case, be due to diminution in concentration of the lactate, for this must remain for long periods above the critical value. Evidence has also been presented to show that accumulation of end products is not the important factor leading to slowing, though temporary piling up of intermediates might play a rôle. Since, in the simplified reaction:

$$\text{Substrate} + O_2(+ \text{ Respiratory Catalyst}) \rightarrow CO_2 + H_2O + \text{End Products},$$

the velocity appears to be independent of substrate, oxygen, or end product concentration, its fall might be attributed to interference with the respiration-catalyzing system. This might mean destruction or out-diffusion (suggested by the fall in Q_{O_2} on dilution) from the cell of one of the key substances in the respiratory chain more rapidly than it is replaced.

The situation with respect to glucose is analogous though quantitatively different; and the ultimate depressant action of methylene blue can also be laid at the door of an injured catalytic system.

Meyerhof (1912) has shown that the oxygen consumption of acetone-yeast is doubled by the addition of methylene blue, whereas that of the intact cells is depressed by it; and similar results were obtained with staphylococci (1917). Barron (1930) likewise has found little or no stimulating action of methylene blue on normal tissues, though it does increase the respiration of cancer and other aerobically glycolysing material. Gerard (1930) has found that medullated nerve, on the contrary, though lacking aerobic glycolysis, is stimulated by methylene blue to a marked respiratory increase, and Chang (unpublished) has confirmed this for non-medullated nerve and with cresyl blue. *Sarcina* in this respect resembles nerve. It may be noted in passing that methylene blue largely or entirely reverses the cyanide inhibition of respiration in the case of staphylococci (Meyerhof) and nerve (Gerard, Chang). There is no evidence that the yellow pigment of *Sarcina* acts in a similar way, though such activity might contribute to the cyanide insensitivity of this organism.

Summary

The Q_{O_2} of washed *Sarcina lutea* in water suspension at 22° C. in the relatively steady state averages 2.5. There is no untoward effect of crowding in heavy suspensions. The presence of NaCl does not modify the Q_{O_2} until $M/5$ concentrations are reached, when a slight depression may result. The Q_{O_2} is also unaffected by pH, at least between 7 and 8, or by the presence of phosphate buffer mixtures.

Glucose addition causes a marked increase in the Q_{O_2}, the maximum being largely independent of glucose concentration. The extra oxygen used under the influence of glucose may be more than that required to fully oxidize it which, with other evidence, suggests a "specific dynamic action."

Sodium lactate may increase the Q_{O_2} in water suspension over twenty-fold. Its addition is always followed by a great rise of the respiratory rate, the maximum reached being independent of lactate concentration, at least between 0.05 and 2.0 per cent. The respiration falls rapidly back to normal after addition of small amounts, and the extra oxygen consumed accounts for full oxidation of the added lactate, both *d* and *l* forms. With larger concentrations of lactate, the increased respiration also falls after the initial maximum, but more slowly. This fall is not primarily due to removal of lactate nor accumulation of end products.

Methylene blue added to a suspension in water doubles respiration at first, later depresses it. Added to one in glucose solution the same sequence appears. The Q_{O_2} in glucose is increased only 50 per cent by the dye, but this is an absolute increase over twice that in water; so that glucose and methylene blue added together to a water suspension cause a greater increase in respiration than the sum of their separate effects. The dye added to lactate solutions seems to be depressant from the start.

Thioglycollic acid doubles the respiration of a buffered water suspension.

Sodium cyanide causes no inhibition of respiration up to concentrations of $M/100$ or somewhat stronger. This is true for the low respiration of suspensions in water, saline, or phosphate buffer and for the increases evoked by methylene blue, lactate and glucose. Extremely strong cyanide concentrations do depress, but even $M/10$ NaCN does not abolish more than 2/3 to 3/4 of the total respiration.

Carbon monoxide containing 5 per cent oxygen has no effect on the respiration of a water suspension, but somewhat inhibits the

increase in glucose and largely that in lactate. Light has little if any effect on the inhibition.

The metabolism of *Sarcina lutea* is compared with that of other cell types.

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